molecules. SMFS has been used to study membrane protein folding inside the protein's native lipid bilayer. Since membrane proteins are inserted vectorially in the lipid membrane in vivo, it is of relevance to probe them by applying a directed force. Mechanically unfolding membrane proteins maps regimes of the energy landscape very different from those mapped in studies with chemical denaturants.
SMFS experiments suggest that the mechanical characteristics of a membrane protein are linked to its functional properties. Changing the experimental conditions like temperature, mutations, or using different metal ions changes the mechanical characteristics of α-helical membrane proteins like bacteriorhodopsin and bovine rhodopsin. Recently, for the first time, we have also probed the mechanics of a β-barrel membrane protein, OmpG. Mechanical unfolding of OmpG shows that β-hairpins are more stable than individual β-strands. Moreover, the changes in the intramolecular interactions of OmpG associated with its pH dependent gating can be correlated with its mechanical properties, e.g., rigidity of certain structural segments.
In summary, I will talk about the importance of mechanical properties of membrane proteins in (un)folding and function.

144-Plat
Temperature Dependence of the High-Pressure Denatured State of Lambda Repressor
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When dropped from 2500 atm to 1 atm in a fast pressure jump experiment, the YG mutant of the five-helix bundle lambda repressor refolds close to the "speed limit", about 40 times faster than observed in a temperature jump experiment. We carried out molecular dynamics simulations to address the question of why refolding from the pressure denatured state is so much faster than refolding upon temperature jump.
The temperature dependence of the high-pressure denatured state of the YG mutant was investigated for this purpose. The high pressure used in the simulation favors denaturation, but also slows down protein dynamics.
High temperature was employed to speed up the dynamics. The high-pressure denatured state was identified as a compact structure with the helices I and IV being the most stable elements. Rapid nucleation of helices I and IV may be a major factor in enhancing the refolding rate once the pressure is dropped to 1 atm.

145-Plat
Protein Unfolding and Refolding by Multidimensional Spectroscopy
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Unfolding and refolding studies using chemical denaturants such as urea or guanidine hydrochloride have contributed tremendously to our understanding of the thermodynamics and kinetics of protein folding and stability. The simplest and most frequently used approach to analyse denaturant-induced unfolding is based on the assumption that the free energy of unfolding in the absence of denaturant can be extrapolated from free energy values measured in the presence of finite denaturant concentrations. However, a major limitation of this approach lies in the large uncertainty inherent in the extrapolation procedure, which results in poor reliability of the best-fit parameter values.
Here we show that this limitation can be overcome by combining multiple spectroscopic signals - including fluorescence, circular dichroism, and absorbance - recorded at many different wavelengths. Modern spectrometers can collect all of these signals in a quasi-simultaneous and fully automated way. We have optimised the number of wavelength values used, the integration time per data point, the increment in the denaturant concentration, and the weighting scheme applied for global data fitting. Compared with the traditional approach based on the use of a single or a few wavelengths, we could thus improve the reliability of the free energy value by an order of magnitude (in terms of the width of the confidence intervals). We exemplify and validate this novel approach using some representative proteins and explain how it can be exploited to quantify subtle changes in protein stability which have thus far remained elusive.

146-Plat
Unzip Single Protein Zippers using Optical Tweezers
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Basic leucine zippers (bZIPs) are dimeric alpha-helical domains found in many transcription factors. They comprise coiled coil regions (zippers) that mediate specific protein dimerization, and basic regions that undergo disorder-to-order transitions to grip their cognate DNA binding sites. It remains unclear how folding of the two regions and binding of DNA are coupled. We investigated the detailed folding kinetics of a model bZIP domain derived from GCN4 transcriptional factor, using high-resolution optical tweezers. When pulled from the same amino- or carboxyl-terminals, the coiled coil alone folds and unfolds rapidly in a two-state manner, with little energy barrier. The folding kinetics of the full GCN4 bZIP domain is similar to the coiled coil alone. However, the presence of its binding site (AP1) induces a third new state corresponding to the bZIP-DNA complex. The probability of this new state critically depends upon the AP1 concentration, which reaches a maximum at an intermediate AP1 concentration. Results from our single-molecule manipulation experiments reveal a complex folding energy landscape even for a small protein domain that can be further tuned by their binding ligands.

147-Plat
Single Molecule Analysis of Protein Folding on the Ribosome
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Most proteins must fold into specific three-dimensional structures to become biologically active. Insights into the mechanisms underlying protein folding have been gained largely from *in vitro* refolding experiments employing isolated full-length proteins. In the cell, however, proteins are synthesized by the ribosome in a vectorial manner. Hence, amino-terminal portions of the protein can participate in folding events while more carboxyl-terminal segments are still being synthesized. As translation proceeds, the elongating nascent protein traverses the polypeptide exit tunnel before emerging from the exit site located at the surface of the large ribosomal subunit. Interactions with components of the tunnel and the area around the exit site have been shown to influence the conformation of the emerging protein. Thus, it is likely that both the vectorial process of translation itself and the ribosomal environment affect the folding pathway of translating polypeptides. We have developed an experimental methodology to directly probe folding transitions of ribosome-associated nascent polypeptides by single molecule force spectroscopy. Using Optical Tweezers, we are able to "pull" on the nascent protein emerging from the ribosome. In combination with a reconstituted translation system, we are capable of interrogating the conformational dynamics of defined, incrementally longer nascent polypeptides. We find that interactions with the ribosome result in an overall deceleration of folding kinetics. The ribosome affects specific transitions during the folding of T4 lysozyme, used as a model protein in our studies. Our results suggest that the ribosome has properties of a molecular chaperone for translating polypeptides, preventing premature folding and maintaining the nascent chain in a folding-competent state. Our experimental system has allowed us to recapitulate the folding transitions during the earliest stages of protein biogenesis and to dissect how the ribosome contributes to the efficient folding of newly synthesized proteins.

PLATFORM L: Voltage-gated K Channels - Gating I

148-Plat
Voltage-Sensor Relaxation in Hv1 Proton Channels
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Hv1 is a voltage-gated and proton-selective channel composed of four helical transmembrane segments (S1-S4) that adopt a voltage-sensor (VS) domain fold that is common to voltage-dependent ion channels and voltage-sensitive phosphatases (VSPs). Unlike other voltage-gated channels, the Hv1 VS also functions as a sensor for the transmembrane pH gradient and contains a water-wire for H^+ conduction. However, despite exhibiting biophysical properties that are specific to its proton channel function, the Hv1 VS appears to share significant structural and functional homology with other VS domains. VS relaxation is manifested by a negative shift in the voltage dependence of sensing charge (Q) movement in response to prolonged depolarization. Previous studies on ciVSP led to the hypothesis that depolarization-induced movement of S4 leads to a rapid distortion of the VS structure. A compensatory voltage-independent conformational transition termed relaxation allows the VS to adopt a more thermodynamically stable state, and is revealed by a negative shift in the Q-V relation. We postulated that because relaxation is likely to represent an intrinsic feature of VS domain function, Hv1 VS would also enter a relaxed state after sustained depolarization. In order to test this hypothesis, we elicited Hv1 opening by applying voltage steps to positive potentials for varying durations and then measured the voltage dependence of channel closure ($V_{0.5}$). As predicted, depolarizing pre-pulses caused $V_{0.5}$ to shift toward negative